# IgE and mast cell responses in collaborative cross mice: Role of *Sp140*

**Authors**

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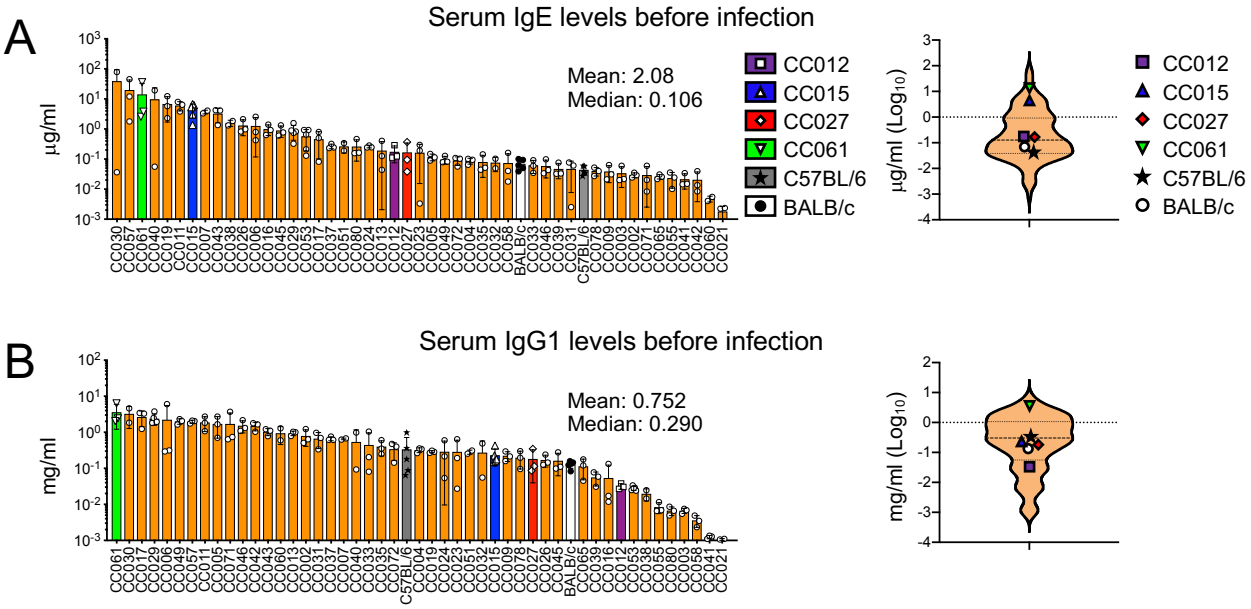
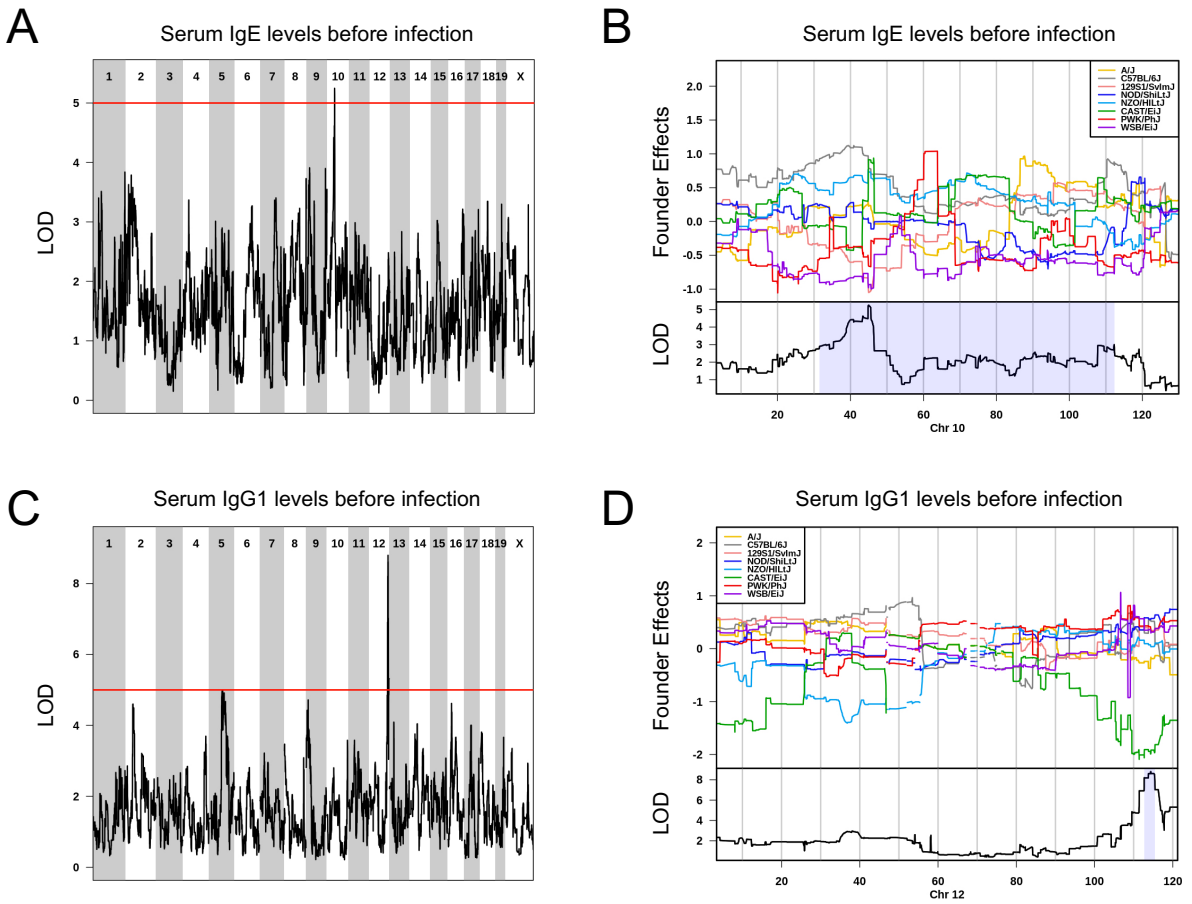
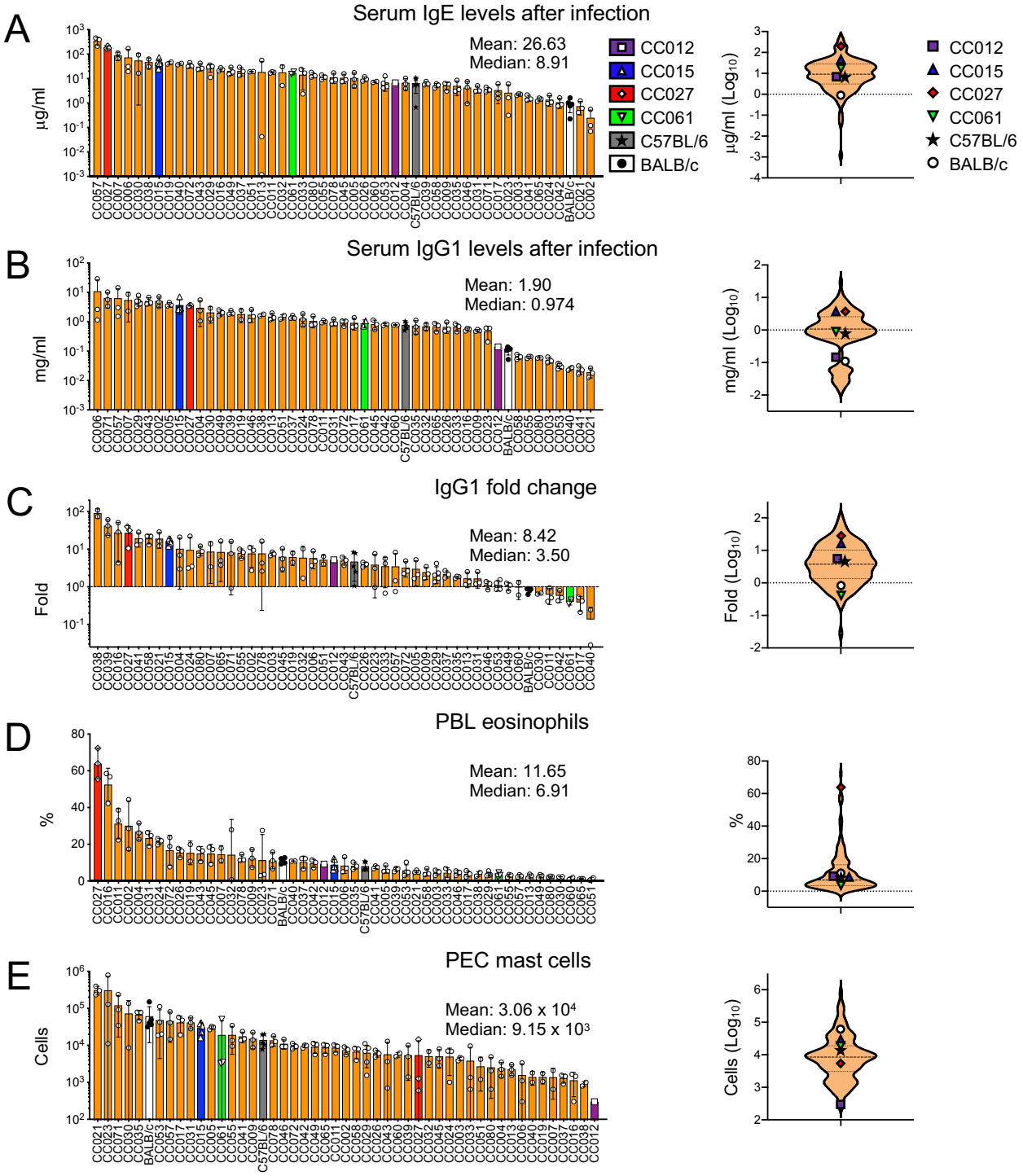
**Supplemental Data**




**Supplemental Figure 1. Baseline levels of IgE and IgG1 in CC, C57BL/6J and BALB/c mice.**
Serum samples were obtained from mice a week before the beginning of PCA (**Figure 1**). Summary graphs (left) and violin plots (right) of baseline levels of serum IgE (**A**) and IgG1 (**B**) for each mouse genotype are shown. n=1–5 mice per genotype (see **Supplemental Table 1**) from eight independent experiments. Purple, blue, red, green, black, and white lines, bars and dots represent CC012, CC015, CC027, CC061, C57BL/6J and BALB/c mice, respectively. Barplots with error bars for summary graphs indicate means ± SDs, and each dot indicates one mouse. Dashed lines in violin plots indicate 25$^{th}$ and 75$^{th}$ quartiles and median.

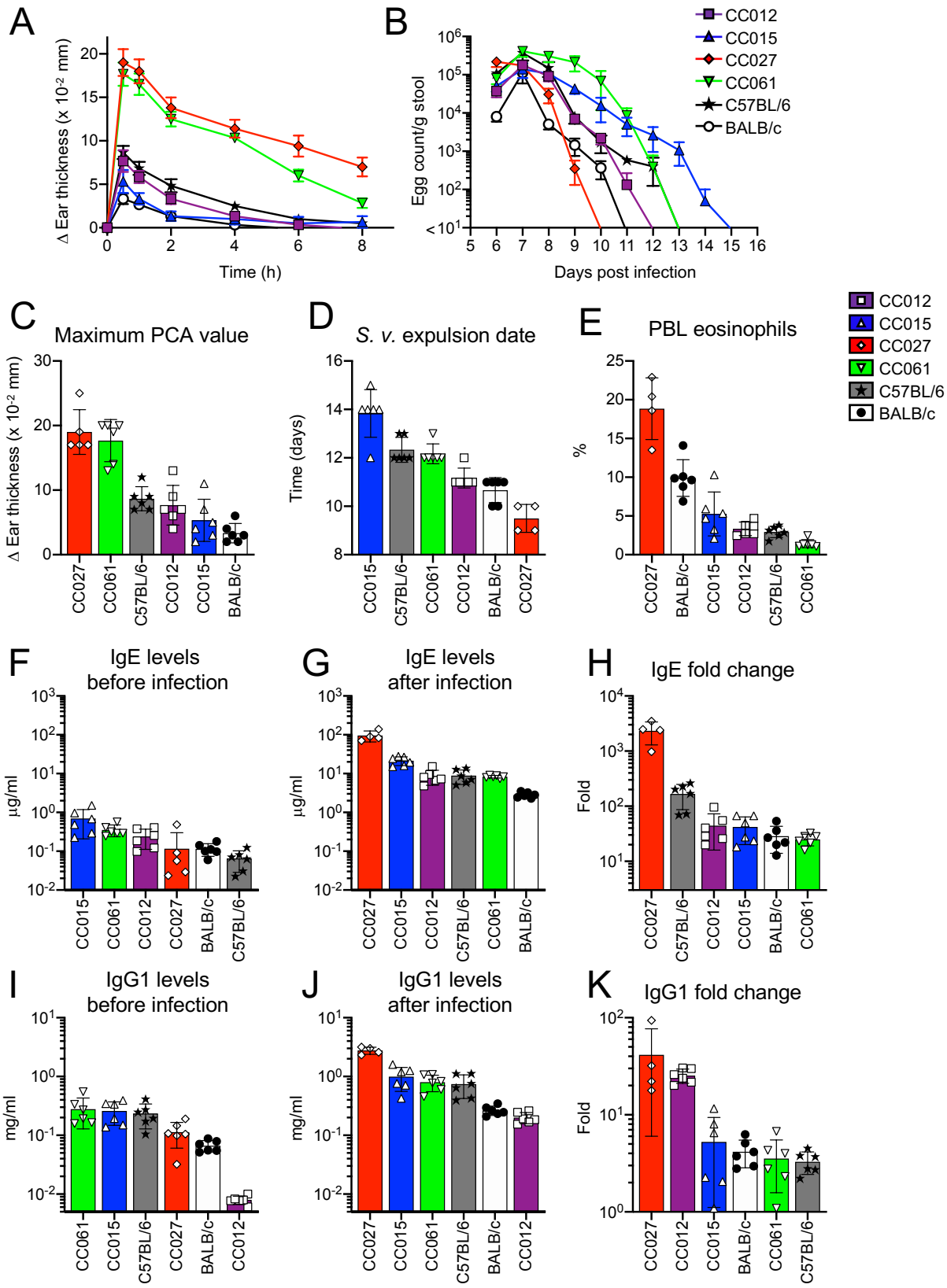



**Supplemental Figure 2. QTL analysis for baseline levels of mouse IgE and IgG1.**
(**A and C**) QTL analysis for chromosomal regions associated with baseline serum levels of IgE (**A**) and IgG1 (**C**). Red lines indicate the LOD score threshold for P = 0.05. (**B and D**) Analysis for founder effects associated with baseline serum IgE levels within chromosome 10 (**B**) and with baseline serum IgG1 levels within chromosome 12 (**D**).



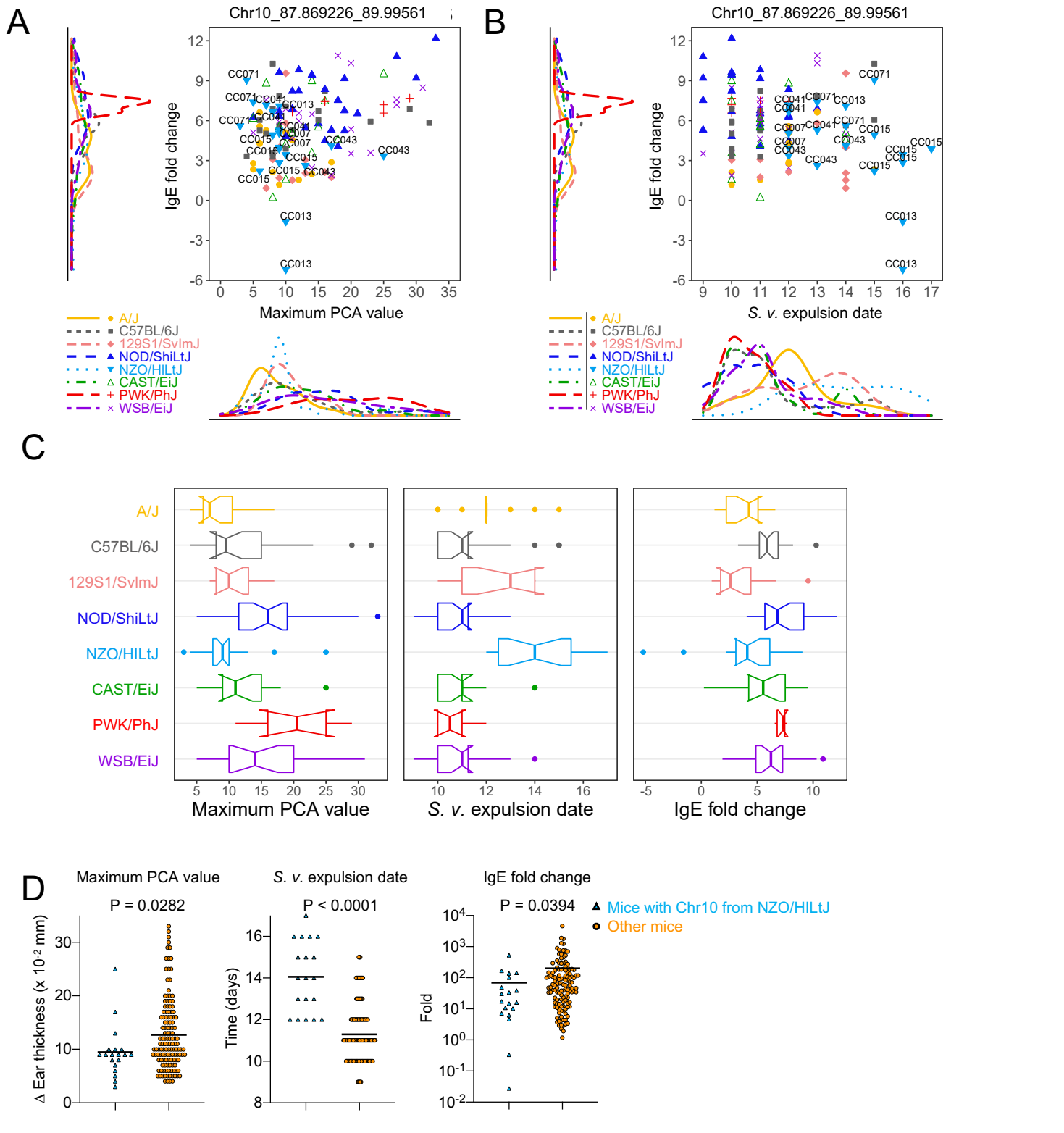

**Supplemental Figure 3. Immunological parameters were assessed after *S. v.* infection in CC, C57BL/6J and BALB/c mice.**

Mice were treated as in **Figure 1A**. All samples were obtained three days after the mice stopped producing *S. v.* eggs. Summary graphs (left) and violin plots (right) of serum IgE levels after *S. v.*-infection (**A**), serum IgG1 levels after *S. v.*-infection (**B**) and the IgG1 fold change from the baseline after infection (**C**), percentage of eosinophils in peripheral blood leucocytes after infection (**D**), and number of mast cells in peritoneal exudate cells (PEC) after infection (**E**) of each genotype of mice are shown. n=1–5 mice per genotype (see **Supplemental Table 1**) from eight independent experiments. Purple, blue, red, green, black, and white lines, bars and dots represent CC012, CC015, CC027, CC061, C57BL/6J and BALB/c mice, respectively. Barplots with error bars for summary graphs indicate means ± SDs, and each dot indicates one mouse. Dashed lines in violin plots indicate 25$^{th}$ and 75$^{th}$ quartiles and median.



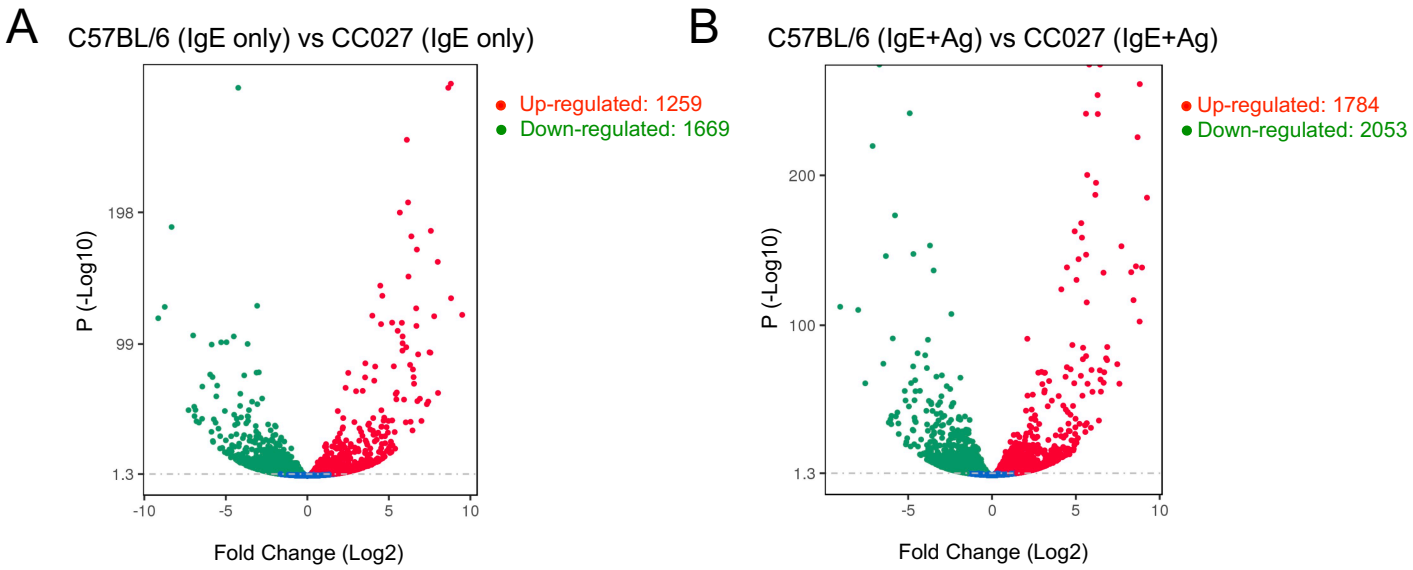

**Supplemental Figure 4. PCA intensity and immunological parameters were evaluated before and after *S. v.* infection in CC, C57BL/6J and BALB/c mice.**

CC012, CC015, CC027, CC061, C57BL/6J and BALB/c mice were treated as in **Figure 1A**. (**A**) PCA reaction. Mean changes (Δ) in ear thickness over time after intravenous injection in each genotype of mice are shown. (**B**) *S. v.* infection. Mean *S. v.* egg numbers excreted after worm inoculation in each genotype of mice are shown. (**C to K**) Summary graphs of the maximum values of PCA (**C**), *S. v.* expulsion date (**D**), percentage of eosinophils in peripheral blood leucocytes after infection (**E**), serum IgE levels before (**F**) and after (**G**) *S. v.*-infection, and the IgE fold change after infection (**H**), serum IgG1 levels before (**I**) and after (**J**) *S. v.*-infection, and the IgG1 fold change after infection (**K**). All samples in E, G and J were obtained three days after the mice had stopped producing *S. v.* eggs. Purple, blue, red, green, black and white lines and bars represent CC012, CC015, CC027, CC061, C57BL/6J and BALB/cJ, respectively. Barplots with error bars indicate means ± SDs, and each dot indicates one mouse. n=4 for CC027 and n=6 for other genotypes from three independent experiments.

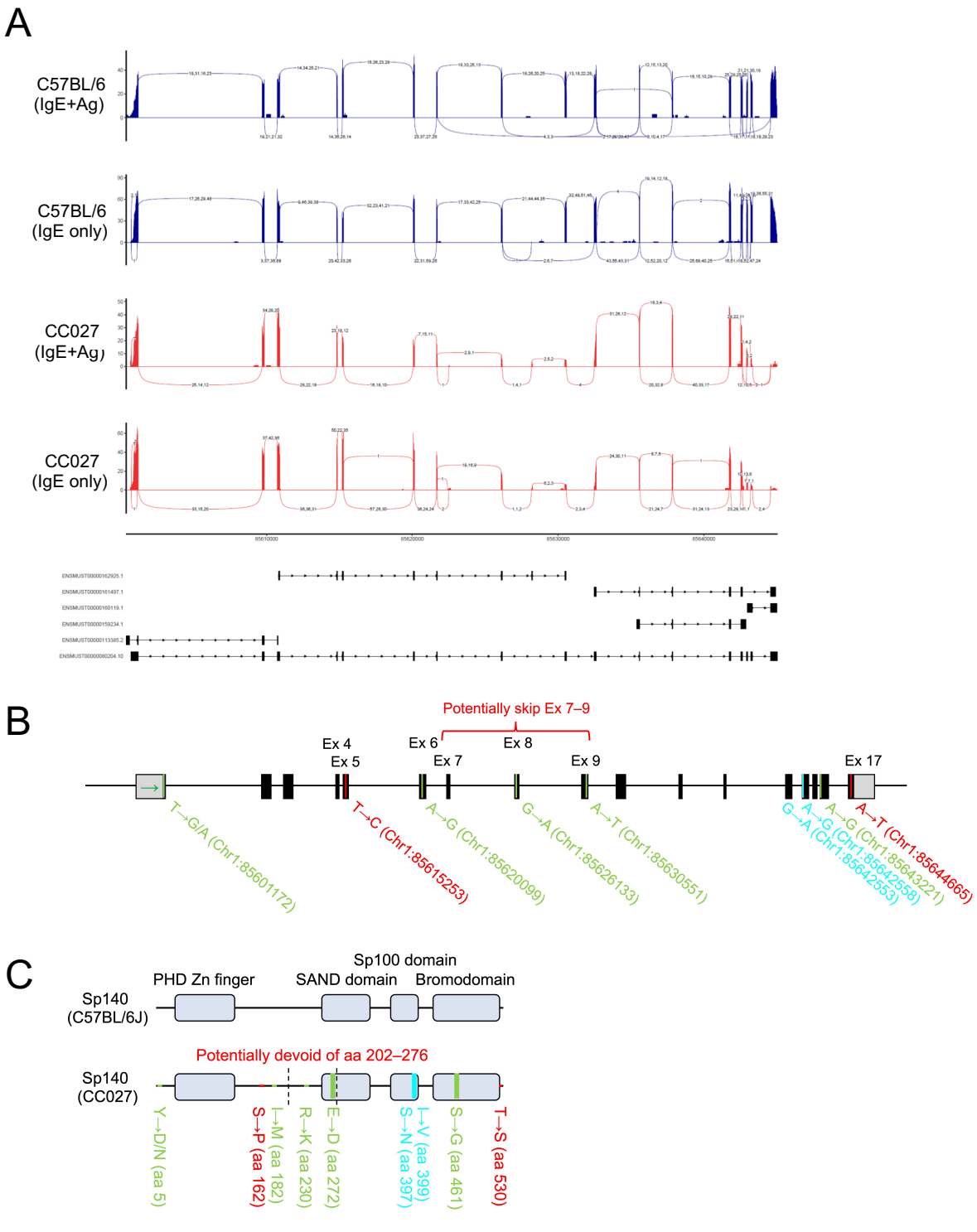



**Supplemental Figure 5. Chromosome 10, region 79.4–94.0 Mb, from NZO/HILtJ associates with weaker MC responses in CC mice.**

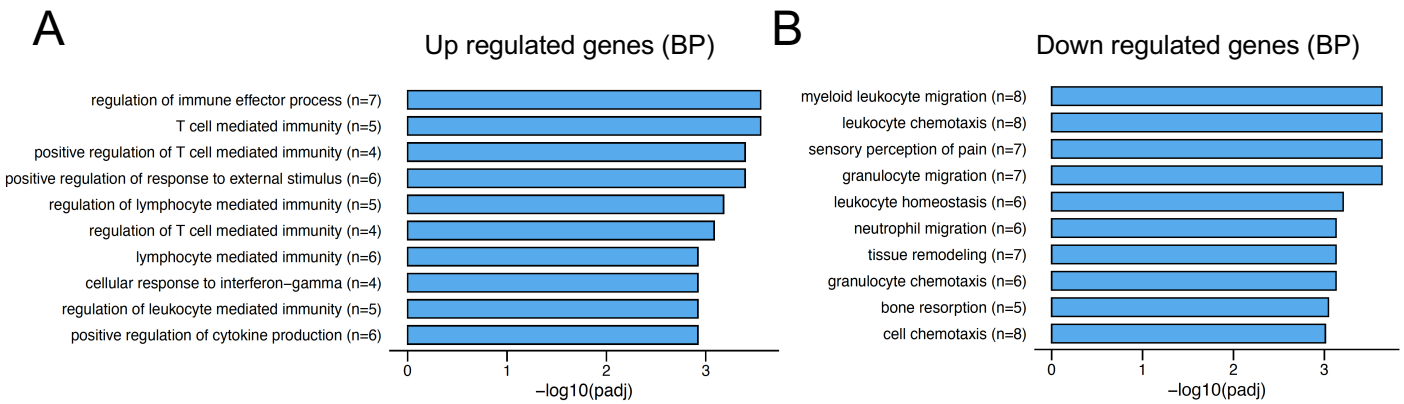
(**A and B**) Inter-correlation among maximum PCA value, *S. v.* expulsion date, and IgE-fold change in CC mice as in **Figure 2**. The founder strain of each CC mouse's chromosome 10, 87.9–90.0 Mbp are indicated in eight different colors (yellow; A/J, brown; C57BL/6J, pink; 129S1/SvImJ, dark blue; NOD/ShiLtJ, light blue; NZO/HILtJ, green; CAST/EiJ, red; PWK/PhJ, purple; WSB/EiJ). (**C**) CC mice were classified into eight groups according to the founders of their chromosome 10, 87.9–90.0 Mbp. Notched boxplots of maximum PCA value, *S. v.* expulsion date, and IgE-fold change of each group are shown. (**D**) Mice were divided into two groups according to their chromosome 10, 87.9–90.0 Mbp being from NOD/ShiLtJ (light blue) or not (orange). Dotplots of maximum PCA value, *S. v.* expulsion date, and IgE-fold change of each group are shown. Each dot indicates one mouse and the bars indicate the means. P values were determined by Student's *t* test.



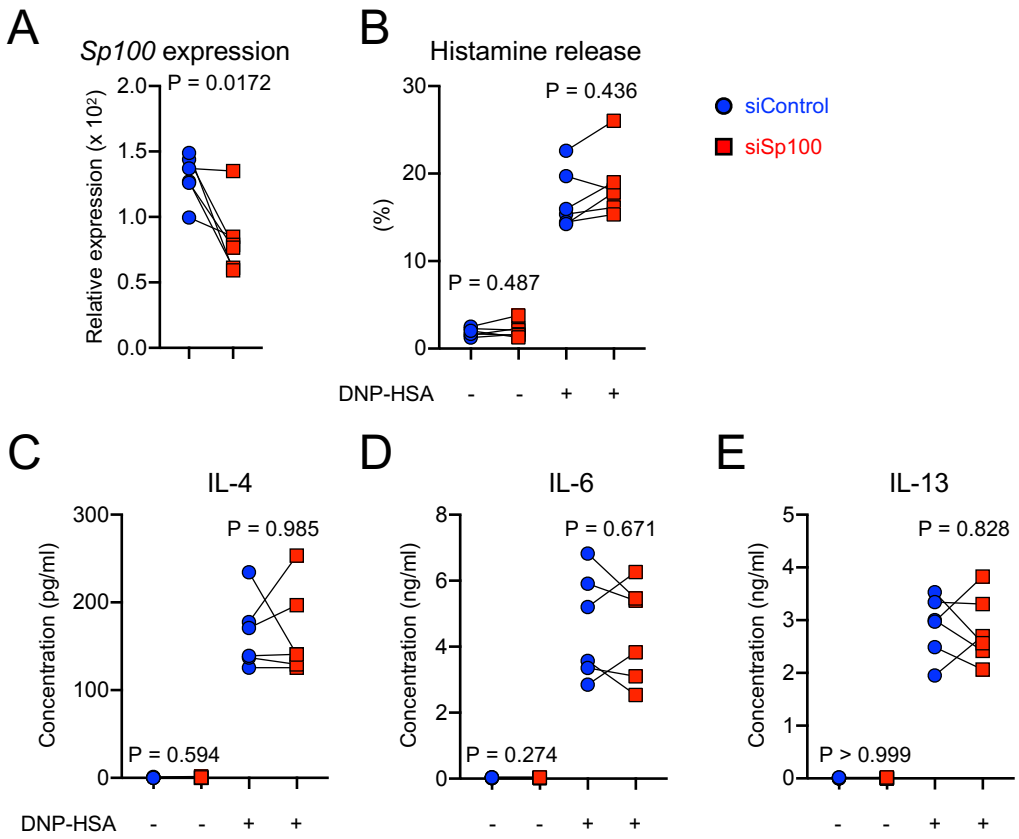

**Supplemental Figure 6. BMCMCs of CC027 and C57BL/6J mice differ in RNA expression profiles.**
BMCMCs from CC027 and C57BL/6J mice were prepared and stimulated as in **Figure 4.** MCs were sensitized with mouse anti-DNP-IgE mAb (1 µg/mL) overnight, then stimulated with (IgE+Ag) or without (IgE only) DNP-HSA (20 ng/mL) for 1 hour. Volcano plots of differentially expressed genes between BMCMCs from CC027 and C57BL/6J mice stimulated without (**A**; IgE only) or with (**B**; IgE+Ag) DNP-HSA.




**Supplemental Figure 7. CC027 mice express *Sp140* devoid of Ex 7–9.**
(**A**) Sashimi plots showing alternative splicing pattern for the entire *Sp140* in C57BL/6J (blue) and CC027 (red) mice. (**B**) Schematic representation of the mouse *Sp140* gene. Nine missense SNPs (red; NOD/ShiLtJ-specific SNPs, green; SNPs not specific for NOD/ShiLtJ, light blue, newly identified SNPs) on CC027 *Sp140* are indicated. (**C**) Schematic representation of the mouse Sp140 protein. The expected Sp140 protein expressed in C57BL/6J and CC027 mice, and the point mutations in CC027 mice caused by SNPs, are shown.




**Supplemental Figure 8. *Sp140*-controlled genes contribute to immune-related processes in CC027 BMCMCs.**
GO biological processes of up- (**A**) and down- (**B**) regulated *Sp140*-controlled genes (mouse macrophages) in non-stimulated CC027 BMCMCs. Genes extracted in **Figure 6I** were subjected to GO pathway analysis.




**Supplemental Figure 9. *Sp100* is dispensable for IgE-dependent MC responses.**
Bone marrow cells from C57BL/6J mice were cultured with IL-3 for 6–7 weeks to generate BMCMCs. Control siRNA or siRNA against *Sp100* was transfected into the cells. (**A**) mRNA level for *Sp100* twenty-four hours after the siRNA transfection was determined by quantitative PCR. The *Sp100* levels are indicated as relative to *Actb*. (**B**) siRNA-transfected cells were sensitized with mouse anti-DNP-IgE mAb (1 μg/mL) overnight, then stimulated with DNP-HSA (10 ng/mL) for 1 hour. Histamine content in cell lysates and culture supernatants was determined by ELISA. Histamine release rates are indicated as % release (supernatants) out of total histamine contents of the cells (cell lysate plus supernatant). (**C to E**) siRNA-transfected cells were stimulated as in (**B**) but for 6 hours. The levels of IL-4 (**C**), IL-6 (**D**) and IL-13 (**E**) in the culture supernatants were determined by ELISA. Each dot indicates different biological replicates. n=6 pooled from two independent experiments. P values were determined by paired Student's *t* test.